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Role of regulatory miRNAs of the Wnt/ β-catenin signaling pathway in tumorigenesis of breast cancer

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Abstract

Breast cancer is the most commonly diagnosed malignancy in women worldwide. Recently, uncontrolled expression of microRNAs was detected in several human disorders like cardiovascular, neurological, intestinal and autoimmunity diseases. MicroRNAs (miRNAs) are now investigated as novel prognostic and diagnostic biomarkers for several solid tumors like breast, lung, and gastrointestinal cancers. Current data suggest that miRNAs are implicated in various oncogenic processes implicated in breast cancer carcinogenesis trough modulating canonical Wnt pathway. Aberrant activation of Wnt/b-catenin signaling was shown to be significantly associated with tumor progression and poor prognosis in patients with breast cancer. This review presents recent findings on the molecular mechanism of microRNAs in regulation of Wnt/β-catenin signaling involved in tumorigenesis of breast cancer.

Keywords: Wnt/β-catenin signaling; breast cancer; MicroRNA

1. Introduction

Breast cancer ranks as the most prevalent malignancy around the world and the second reason of cancer related death in female population [1]. Despite great improvements in screening, diagnosis and therapeutic strategies, the prognosis and survival outcomes in patients with breast cancer are still unsatisfying [2]. Therefore, it is clinically desirable to identify novel prognostic markers in breast cancer. Recent studies revealed that breast cancer as a heterogeneous disease can be divided into several molecular subtypes with different pathological features and clinical responses [3, 4]. The breast cancer tumorigenesis was recognized as a multistep process which is modulated by various oncogenic cell signaling cascades including PI3K/AKT and Wnt/b-catenin pathways [5-9]. For instance, upregulation of b-catenin and its downstream targets were shown to be significantly related to malignant features and poor clinical outcomes in patients with breast cancer [10, 11]. Recent molecular investigations to identify novel mechanisms involved in breast cancer tumorigenesis indicate that some miRNAs were upregulated along cancer development leading to aberrant activation of various oncogenic signaling pathways.

MicroRNAs (miRNAs) are endogenous, small non-coding RNAs, that are involved in regulating gene expression by pairing with 3'-untranslated regions in target mRNAs. Aberrant expression of multiple miRNAs is considered to have critical roles in controlling various biological events like cell differentiation, metabolism, proliferation, tumorigenesis and drug resistance [12-14]. Regarding their target genes, miRNAs can act as tumor-suppressors or oncogenes in modulating intracellular signalings events. It is well stablished that the over-expressed miRNAs have prominent effects on tumor cell growth and progression by activating multiple oncogenic signalings like Wnt/β-catenin pathway [15, 16]. The oncogenic activity of these miRNAs is mediated by targeting several tumor suppressors including GSK-3 β, APC and PTEN resulted in tumor growth and metastasis in breast cancer patients. Conversely, the tumor suppressor

miRNAs were shown to have anti-cancer role by inhibiting tumor cell proliferation through suppressing β-catenin or Wnt receptors like frizzled proteins.

The regulatory function of miRNAs on the Wnt/β-catenin signaling, suggesting a promising role of miRNAs in breast cancer therapy. Here we reviewed recent findings on the regulatory effects of miRNAs on Wnt/β-catenin signaling involved in tumorigenesis of breast cancer.

2. Wnt/ β-catenin signaling pathway

The Wnt/ β-catenin signaling plays essential roles in cancer cell growth, survival and metastasis [17, 18]. Generally, the intracellular Wnt signaling subdivided into β-catenin dependent (canonical) and β-catenin in-dependent (non-canonical) routes. The canonical Wnt/β-catenin axis was found to be associated with several oncogenic events including tumor cell proliferation, migration, EMT and invasion [18, 19]. In the condition that canonical Wnt signaling is inactivated, the cytoplasmic β-catenin proteins were phosphorylated by tumor suppressors glycogen synthase kinase 3 β (GSK-3 β) and casein kinase 1 (CK1) leading to ubiquitination and proteolysis by proteasomes resulted in inactivation of β-catenin signaling [20]. However, the canonical Wnt signaling was shown to be activated trough binding of Wnt ligand to its specific receptors including LRP-5/6 (LDL receptor–related protein 5 or 6) and Frizzled proteins. This in turn induces next components of Wnt pathway comprising disheveled resulted in deactivation of GSK-3 β and dissociation of β-catenin proteins from destruction complex formed by GSK-3 β, CK1, Axin, and APC (adenomatous polyposis coli). Therefore, β-catenin proteins accumulated in the cytosol can transferred to the nucleus and regulate multiple downstream genes like c-Myc and cyclin D1[21, 22].

Cyclins, which are considered as key promoters of cellular proliferation, have a prominent role in cell cycle progression through inducing cyclin dependent kinase enzymes [10, 23]. Nowadays, it is well-known that any defect in regulation of β-catenin target genes may causes un-controlled cell cycle progression and development of several types of human tumors like

breast, lymphoma, melanoma, colorectal, and prostate cancers [24-27]. Moreover, prolonged activity of Wnt/β-catenin signaling was shown to be associated with clinical poor outcomes and resistance to chemotherapy [28, 29].

3. Regulatory role of oncogenic miRNAs in Wnt/β-catenin signaling in breast cancer cells

It is widely accepted that over-activation of Wnt/β-catenin signaling is implicated in tumorigenesis and progression of multiple human cancers [18]. Growing body of evidence indicate that downregulation of tumor suppressors involved in regulation of Wnt/β-catenin pathway have critical roles in breast cancer tumorigenesis. As shown in figure 1, there are various studies demonstrated that some miRNAs can promote Wnt signaling trough suppressing negative regulators such as GSK-3 β and APC [30]. Recently, miR-1229 was detected to be upregulated in breast tumor patients. Aberrant expression of miR-1229 induces Wnt/β-catenin signaling trough targeting GSK-3 β, APC and ICAT proteins [30]. ICAT also known as inhibitor of β-catenin and TCF (T cell factor), inhibits the expression of downstream targets of Wnt signaling by preventing interaction of β-catenin and TCF. The overexpression of miR-1301 was found to be associated with aggressive features and poor clinical outcomes in patients with breast cancer. It has been demonstrated that the miR-1301 can downregulates ICAT expression by directly binding to 3´-UTR of its mRNA [31]. These data lead to considering miR-1301 as a novel prognostic biomarker for breast tumor. MiR-125b is another Wnt/ β-catenin signaling pathway regulatory miRNA, which is overexpressed in breast cancer [32]. It has been shown that the 3´-UTR of APC mRNA have targeted by miR-125b providing the mechanism by which miR-125b can induce Wnt/ β-catenin signaling by targeting APC. Downregulation of miR-125b was found to be correlated with inhibition of Wnt/ β-catenin signaling resulted in decreased tumor development and dissemination [32]. Thus, repression of miR-125b or overexpression of APC may be considered as potential therapeutic targets for management of breast tumors.

The expression of GSK-3 β protein was also found to be inhibited by miR-3646. Moreover, elevated levels of miR-3646 in breast cancer patients was shown to be associated with both docetaxel resistance and upregulation of β-catenin [33]. To further investigate the mechanism of miRNAs in drug resistance in breast cancer, Shen et al. demonstrated that miR-29a was significantly upregulated in Adriamycin-resistant MCF-7 cells [34]. Ectopic expression of miR-29a was observed to be related to downregulation of GSK-3 β and PTEN tumor suppressors in MCF-7 breast cancer cells. Therefore, blockade of PTEN/GSK-3 β signaling may resulted in Adriamycin resistance in breast tumors. Taken together, these results indicate that miR-3646 and miR-29a can induce drug resistance in breast tumor cells by promoting Wnt/β-catenin signaling trough suppression of GSK-3 β.

Similarly, Ma et al. indicated that miR-301 induces Wnt signaling by targeting PTEN (phosphatase and tensin homolog) in breast cancer cells [35]. PTEN was identified as a master regulator of several oncogenic pathways including PI3K/AKT and Wnt signaling which is downregulated in various human cancers [9, 36, 37].

Additionally, previous findings demonstrated that certain miRNAs can induce canonical Wnt pathway trough targeting Wnt antagonists like Wnt inhibitory factor-1 (WIF1). WIF1 was shown to be repressed via miR-374a which is upregulated in patients with metastatic breast cancer [38]. The overexpression of miR-374a was reported to be strongly associated with tumor growth and reduced survival in breast cancer patients with distant metastases. To further elucidate the metastatic function of miR-374a, it has been found that miR-374a downregulates the expression of Wnt5a in breast cancer cells [38]. The Wnt5a as one of the main regulators of non-canonical pathway, activates CK1 leading to phosphorylation of β-catenin and consequently inhibition of Wnt pathway [39, 40]. These data provide evidence that the oncogenic function of miR-374a in inducing cancer cell metastasis can be mediated by suppressing Wnt/ β-catenin signaling inhibitors. To further explore whether oncogenic miRNAs can inhibit regulatory proteins of Wnt/ β-catenin signaling, Ren et al. reported that miR-638 is upregulated in breast cancer and

inhibits DACT3 as one of the main modulators of Wnt/ β-catenin pathway [41]. DACT3, a member of DACT family, was identified as a potent tumor suppressor which inhibits tumor cell proliferation by regulating β-catenin expression in various human malignancies [42]. Additionally, it has been reported that upregulation of miR-638 was directly correlated with malignant features of breast cancer while regulates cellular autophagy [41].

In another investigation, Chen et al. demonstrated that aberrant expression of miR-373 has prominent role in promoting EMT by inducing mesenchymal markers like β-catenin in breast cancer cells [43]. These results suggest that miR-373 can promote epithelial-to-mesenchymal transition (EMT) and metastasis in breast tumors by upregulating Wnt/ β-catenin signaling pathway.

4. Regulatory role of tumor suppressor miRNAs in Wnt/ β-catenin signaling in breast cancer cells

β-catenin as a potent oncogene is upregulated in multiple human cancers. β-catenin regulates expression of multiple proteins involved in cell proliferation like cyclin D1 which is found to be upregulated in nearly 50% of patients with breast cancer [10]. Aberrant activity of β-catenin was directly related to malignant manifestations and poor prognosis in breast cancer [10, 44]. β-catenin was identified as target gene for some regulatory miRNAs including miR-26b, miR-135, miR-214, miR-216a, and miR-340 that are downregulated in breast tumor cells [45-49]. More investigations on the mechanism of miR-135 was also demonstrated that this tumor suppressor miRNA can inhibit tumor cell proliferation and EMT by regulating GSK3- β and matrix metalloproteinase (MMP) enzymes respectively [46]. Consistently, Xiong et al. reported that miR-30a-5p represses β-catenin downstream targets like cyclin B1, cyclin D1 and c-Myc in breast tumor cells [50]. It has been also reported that expression of miR-30a-5p is inversely related to the expression of ubiquitin protein ligase E3C (UBE3C) in breast cancer cell lines. UBE3C was

shown to be upregulated in breast cancer leads to overexpression of c-Myc and cyclin D1 [50, 51].

Furthermore, miR-138 and miR-449b-5p were identified as tumor suppressor miRNAs which inhibit tumor progression and metastasis by targeting CREPT (Cell Cycle-Related and Expression-elevated Protein in Tumor) [52, 53]. The overexpression of CREPT was detected in breast tumor cells and tissues. CREPT as an oncogene protein, has a critical role in cellular proliferation trough modulating cell cycle-related genes like cyclin D1 [54, 55]. MiR-384 is another tumor suppressor microRNA which is downregulated in breast cancer patients [56]. Upregulation of miR-384 inhibits ACVR1 (Activin A receptor type 1) resulted in repression of Wnt/β-catenin axis in breast tumors. Previous data indicate that ACVR1 can induce tumor cell metastasis by promoting Wnt/β-catenin pathway in several human malignancies like breast cancer [57, 58]. Consistent with these findings, it has been shown that miR-30 inhibits cancer cell invasion and metastasis while induces cell apoptosis by repressing CTHRC1 (Collagen triple helix repeat containing-1) in breast tumors [59]. CTHRC1 is a secretory protein involved in stabilizing binding of Wnt ligand to their Frizzled receptors leading to activation of Wnt signaling pathway. Further studies on the oncogenic function of CTHRC1 indicate that elevated levels of CTHRC1 induces GSK3 β and β-catenin activities which are associated with aggressive clinicopathological manifestations and worse prognosis in several malignant tumors including gastrointestinal, liver, breast and non-small cell lung cancers [60-62]. MiR-140-5p is another Wnt inhibitory miRNA which is down-regulated in breast cancer stem cells. Ectopic expression of miR-140-5p represents anti-tumor activity mediated by targeting Wnt1 [63].

To further investigate the inhibitory function of regulatory miRNAs on Wnt/ β-catenin axis, it has been reported that some miRNAs can limit tumor progression by targeting Wnt receptors like Frizzled proteins. For instance, Liu et al. reported that miR-224 represents anti-cancer properties trough targeting Frizzled 5 receptor proteins resulted in inhibition of Wnt signaling in breast cancer [64]. In line with this, Leu et al. demonstrated that miR-1 inhibits Frizzled 7 and

tankyrase 2 proteins resulted in reduced cellular proliferation and migration in breast tumors [65]. Similarly, Jiang et al illustrated that miR-100 which is repressed in breast cancer patients can inhibit Wnt/ β-catenin pathway by directly targeting Frizzled 8 receptor proteins [66]. In another study, the effective role of regulatory miRNAs on secreted Frizzled-related proteins (SFRPs) was investigated in breast tumor cells. Their results indicate that miR-27a can restrict tumor cell proliferation, migration and invasion by targeting SFRPs [67]. These results support the idea that Frizzled receptors and their related proteins may be considered as promising targets for breast cancer therapy.

To further identify the oncogenic targets regulated by miRNAs, it has been shown that cyclin dependent kinase enzyme 8 (CDK8) has been downregulated by certain miRNAs including miR-107 and miR-26b [45, 68]. The CDK8 enzyme has critical role in promoting cell cycle progression and cellular proliferation resulted in tumor growth and development.

5. Conclusion

Recent data indicate that multiple miRNAs are differentially expressed in breast cancer, and have critical roles in tumor cell proliferation, invasion, and metastasis (Table 1 and 2).

As shown in figure 2, the overexpression of various miRNAs including miR-1229, miR-125b, miR-3646, miR-29a, and miR-301 might contribute to aberrant activation of the Wnt/ β-catenin signaling. The oncogenic activity of these miRNAs is mediated by targeting several tumor suppressors including GSK-3 β, APC and PTEN. Consistently, the Wnt signaling downstream target genes were upregulated resulted in tumor growth, metastasis and drug resistance in breast cancer patients. Conversely, the tumor suppressor miRNAs including miR-26b, miR-135, miR-214, miR-216a, and miR-340 were found to have downregulated in breast cancer patients. These miRNAs were shown to have anti-tumor activity by inhibiting cancer cell proliferation through suppressing β-catenin. These data in combination with recent findings demonstrate that

uncontrolled activity of oncogenic Wnt/ β-catenin signaling is associated to poor prognosis and aggressive behavior in patients with breast cancer.

In this review, we briefly discussed the previous data about the function of Wnt axis regulatory miRNAs involved in carcinogenesis of breast cancer. At present, there is a growing trend on targeting oncogenic miRNAs by certain inhibitors for anti-cancer approaches. Considering the oncogenic or anti-tumor function of miRNAs and their target genes, it has been proposed that miRNAs may be considered as new diagnostic and prognostic biomarkers for breast cancer. Therefore, regulating the oncogenic miRNAs by specific inhibitors or upregulation of anti-tumor miRNAs may be considered as novel miRNA-based therapeutic strategies for management of breast cancer.

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Figures

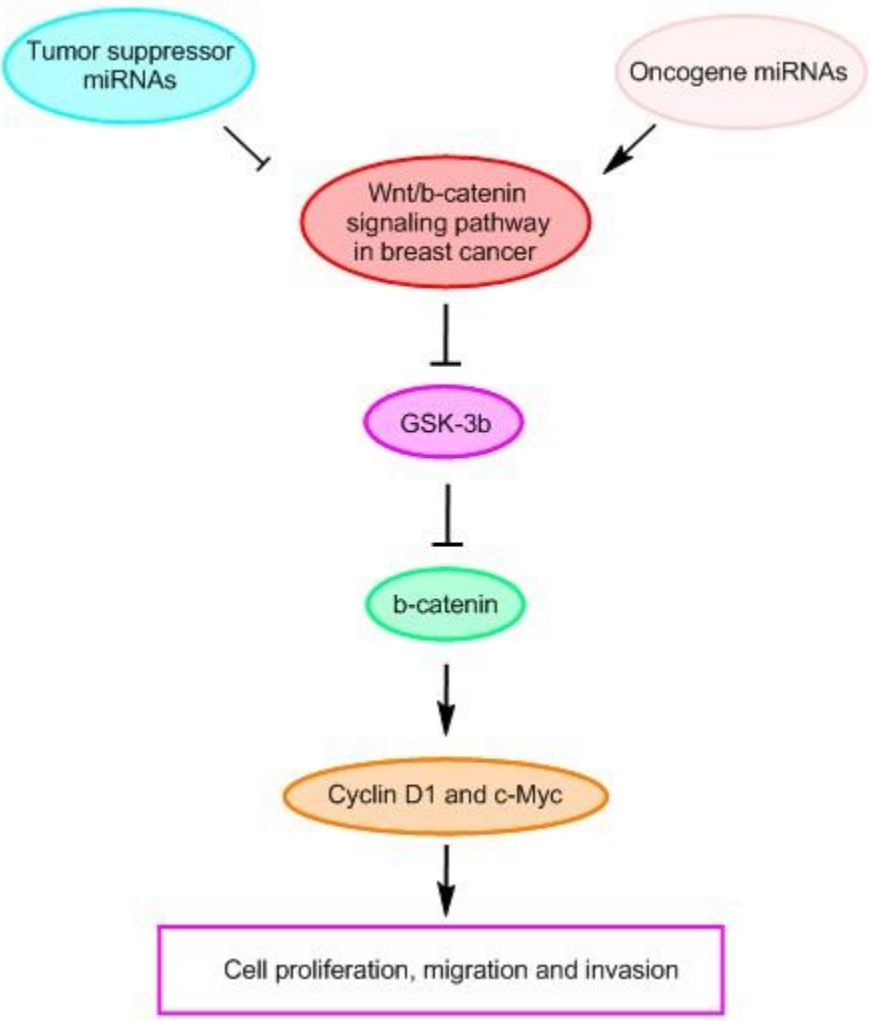

Figure 1) Schematic model of the role of Wnt/β-catenin signaling regulatory microRNAs in breast cancer tumorigenesis.

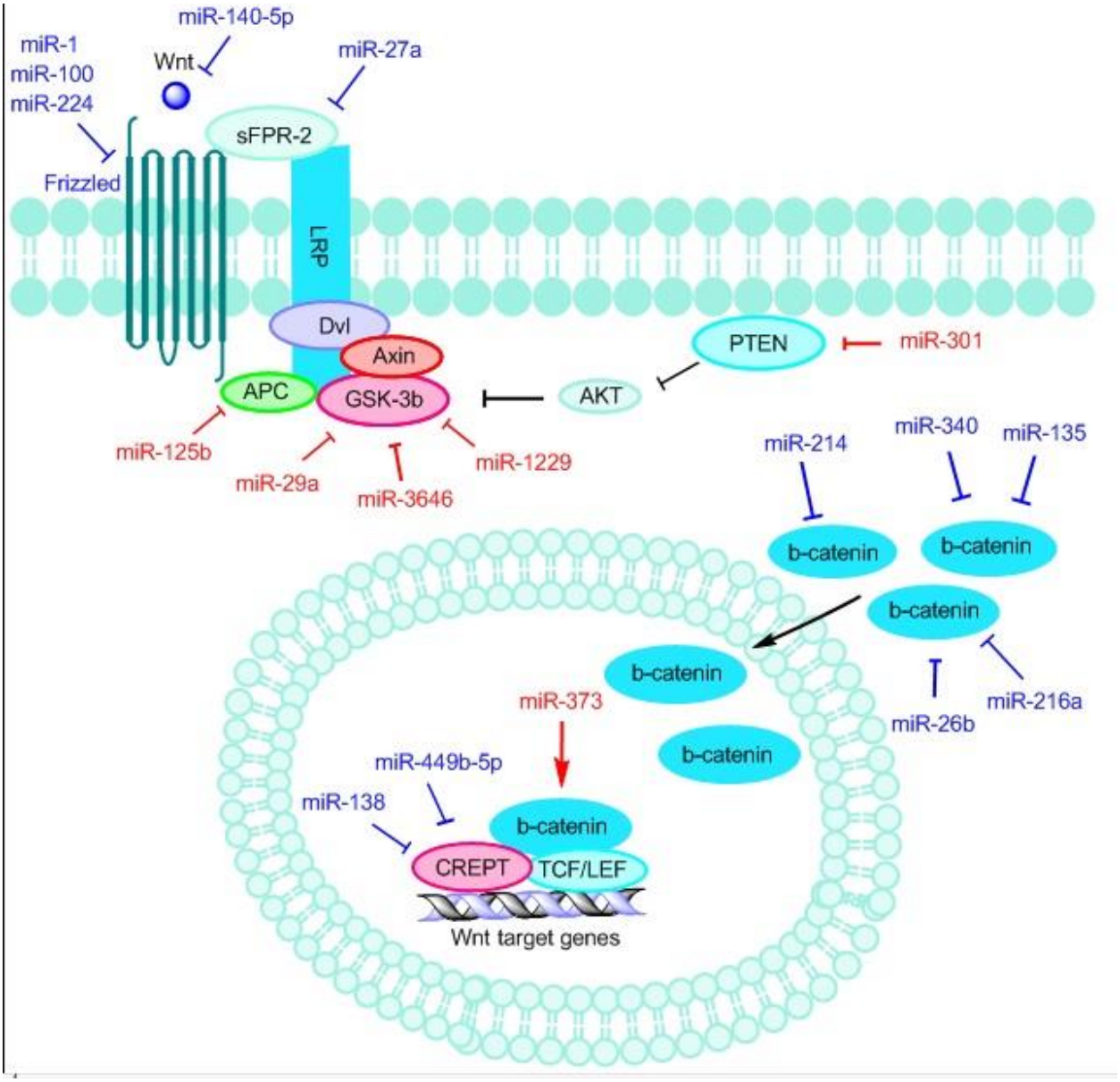

Figure 2) The mechanisms of Wnt/ β-catenin signaling regulatory miRNAs in breast cancer carcinogenesis.

Table1. Oncogenic miRNAs regulate the pathogenesis of breast cancer by targeting the certain components of the Wnt/β-catenin signaling pathway.

microRNA	Molecular alteration	Target	Function	Reference
miR-1229	Upregulation	GSK-3 β, APC, and ICAT	Cellular proliferation	Tan et al., 2016
miR-1301	Upregulation	ICAT	Cellular proliferation	Lin et al., 2016
miR-125b	Upregulation	APC	Cellular proliferation and metastasis	Nie et al., 2019
miR-3646	Upregulation	GSK-3 β	Drug resistance	Zhang et al., 2016
miR-29a	Upregulation	GSK-3 β	Drug resistance	Shen et al., 2016
miR-301	Upregulation	PTEN	Cellular proliferation and metastasis	Ma et al., 2014
miR-374a	Upregulation	WIF1 and Wnt5a	Tumor metastasis	Cai et al., 2013
miR-638	Upregulation	DACT3	Cellular proliferation	Ren et al., 2017
miR-373	Upregulation	β-catenin	EMT	De Chen et al., 2015

Table2. Tumor suppressor miRNAs regulate the pathogenesis of breast cancer by targeting the certain components of the Wnt/β-catenin signaling pathway.

microRNA	Molecular alteration	Target	Function	Reference
miR-26b	Downregulation	β-catenin	Inhibits cellular proliferation	Li et al., 2014a
miR-135	Downregulation	β-catenin	Inhibits cellular proliferation	Jiang et al., 2019a
miR-214	Downregulation	β-catenin	Inhibits cellular proliferation	Yi et al., 2016
miR-216a	Downregulation	β-catenin	Inhibits cellular proliferation	Xie et al., 2019
miR-340	Downregulation	β-catenin	Inhibits cellular proliferation	Mohammadi-Yeganeh et al., 2016
miR-30a-5p	Downregulation	UBE3C	Inhibits cellular proliferation and metastasis	Xiong et al., 2019
miR-138	Downregulation	CREPT	Inhibits tumor growth and metastasis	Liang et al., 2017
miR-449b-5p	Downregulation	CREPT	Inhibits tumor growth and metastasis	Jiang et al., 2019
miR-384	Downregulation	ACVR1	Inhibits tumor metastasis	Wang et al., 2018
miR-30	Downregulation	CTHRC1	Inhibits cell invasion and metastasis	Lai et al., 2017
miR-140-5p	Downregulation	Wnt1	Inhibits cellular proliferation	Wu et al., 2019
miR-224	Downregulation	Frizzled 5	Inhibits tumor progression	Liu et al., 2016
miR-1	Downregulation	Frizzled 7	Inhibits tumor progression	Liu et al., 2015
miR-100	Downregulation	Frizzled 8	Inhibits tumor progression	Jiang et al., 2016
miR-27a	Downregulation	SFRPs	Inhibits cell growth and invasion	Kong et al., 2017
miR-107	Downregulation	CDK8	Inhibits cellular proliferation and metastasis	Li et al., 2014
miR-26b	Downregulation	CDK8	Inhibits cellular proliferation and metastasis	Li et al., 2014